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Rapid Report

Success of Transmyocardial Laser Revascularization Is Determined by the Amount and Organization of Scar Tissue Produced in Response to Initial Injury: Results of Ultraviolet Laser Treatment

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Background and Objective: **Previous studies of transmyocardial laser revascularization have reported open channels after ultraviolet laser treatment and closed channels with infrared lasers. We speculated that differences in long-term channel patency were determined by the healing response to injury.**
Methods: **Channels were made in rat hearts with a frequency-tripled neodymium:YAG laser, at 5 and 10 mJ per pulse, by advancing an optic fiber through the myocardium, from the epicardium to the ventricular cavity. Several months later, we challenged the ability of the channel to supply blood by arterial occlusion and examined the channel structure with polarized light microscopy.**
Results: **Low-pulse energy was associated with lower patency, more fibrosis, and larger infarcts than was the higher energy. Open channels were surrounded by collagen fibers aligned parallel to the channel; in closed channels, fibers were aligned perpendicular to the original channel direction.**
Conclusion: **The amount of initial injury and its repair determine channel patency and function. Lasers Surg. Med. 24:253–260, 1999.**

Key words: ablation; collagen; ischemia; myocardial infarction

INTRODUCTION

Laser-made myocardial channels are used to treat patients who are unsuitable candidates for bypass surgery or angioplasty. Although treatment produces marked reduction in chest pain [1], the procedure remains controversial because the mechanism of action is unknown. It was originally proposed that channels immediately permit blood flow directly from the ventricular cavity; however, studies have reported that channels become blocked, first by thrombus and later by scar [2,3]. The infrared lasers used in these studies create channels by vaporizing tissue water, which causes thermal damage to surrounding muscle. Our hypothesis was that the healing response to such thermal injury plays a role in channel clo-

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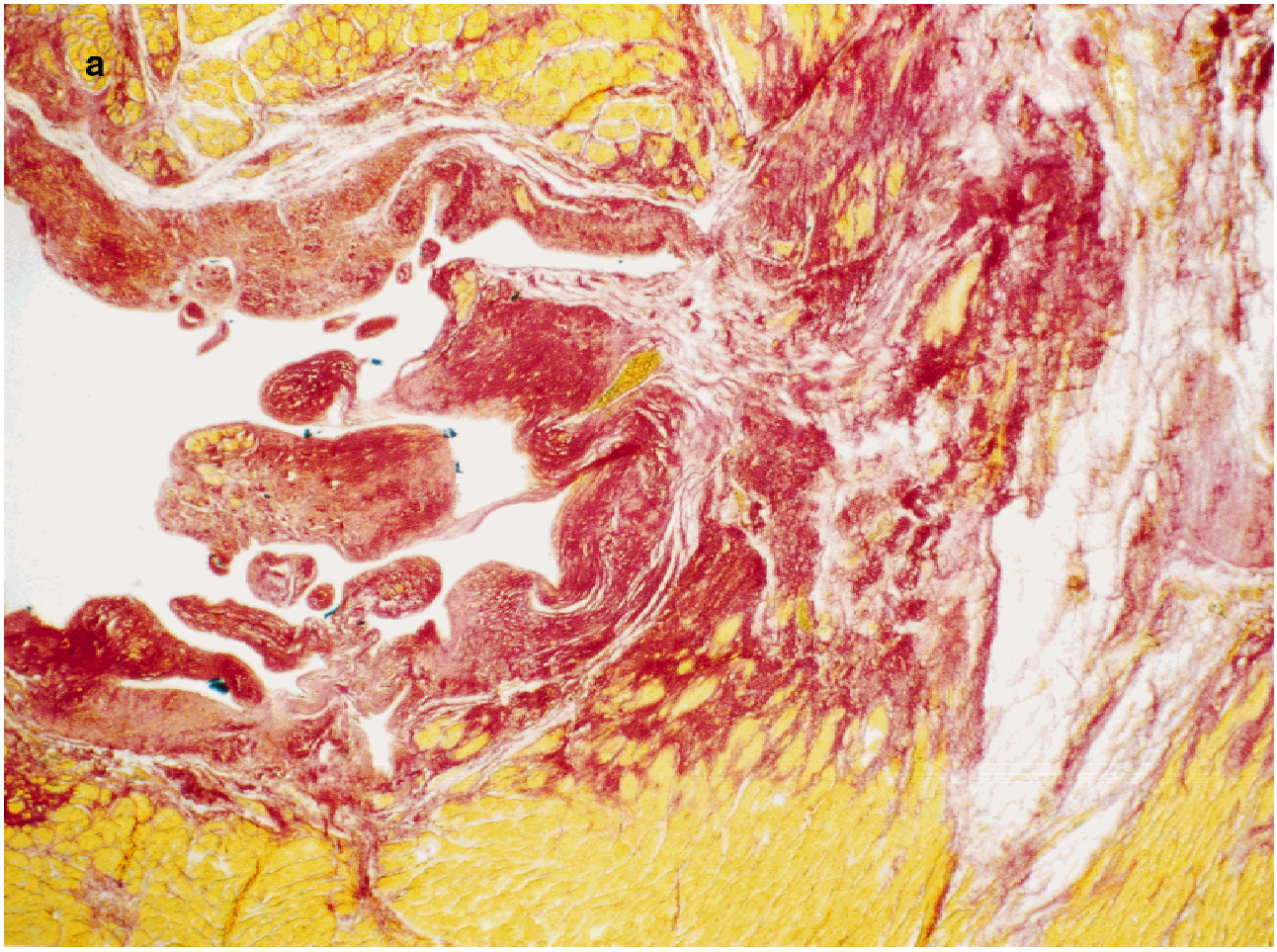

Fig. 1. Open laser channels. Sections were stained with picrosirius red and viewed in brightfield microscopy; muscle appears yellow and collagen appears red. **a:** Extensive scar tissue surrounding two channels made close together.

sure. This concept was supported by a study in which channels made in sheep hearts with an excimer laser were found to be open one month later [4]. Ultraviolet lasers, such as the excimer, produce ablation by the direct absorption of photons by chemical bonds and thus generally cause less thermal injury than do infrared lasers to adjacent tissue [5]. We tested the hypothesis by using another ultraviolet laser, a frequency-tripled neodymium:YAG, at two doses selected to produce channels surrounded by different amounts of injury. We found that, 2–5 months after channel making in rat hearts, only open channels surrounded by small amounts of scar protected against an ischemic challenge. Furthermore, collagen fibers adjacent to open channels were aligned parallel to the channel; however, in closed channels, fibers were aligned perpendicular to the original channel direction. Thus, the amount of injury caused by channel making and its repair appear crucial to the long-term outcome.

METHODS

The protocol used was approved by the hospital's Animal Care and Use Committee and conforms to the principles of the American Physiologic Society. The Heart Institute, Good Samaritan Hospital is accredited by the American Association for Accreditation of Laboratory Animal Care.

Laser Description

We used a frequency-tripled neodymium:YAG laser (λ = 355 nm; Laser Photonics, Orlando, FL) to create channels at two energies: 5–6 mJ and 9–10 mJ per pulse, both at 20 Hz and a

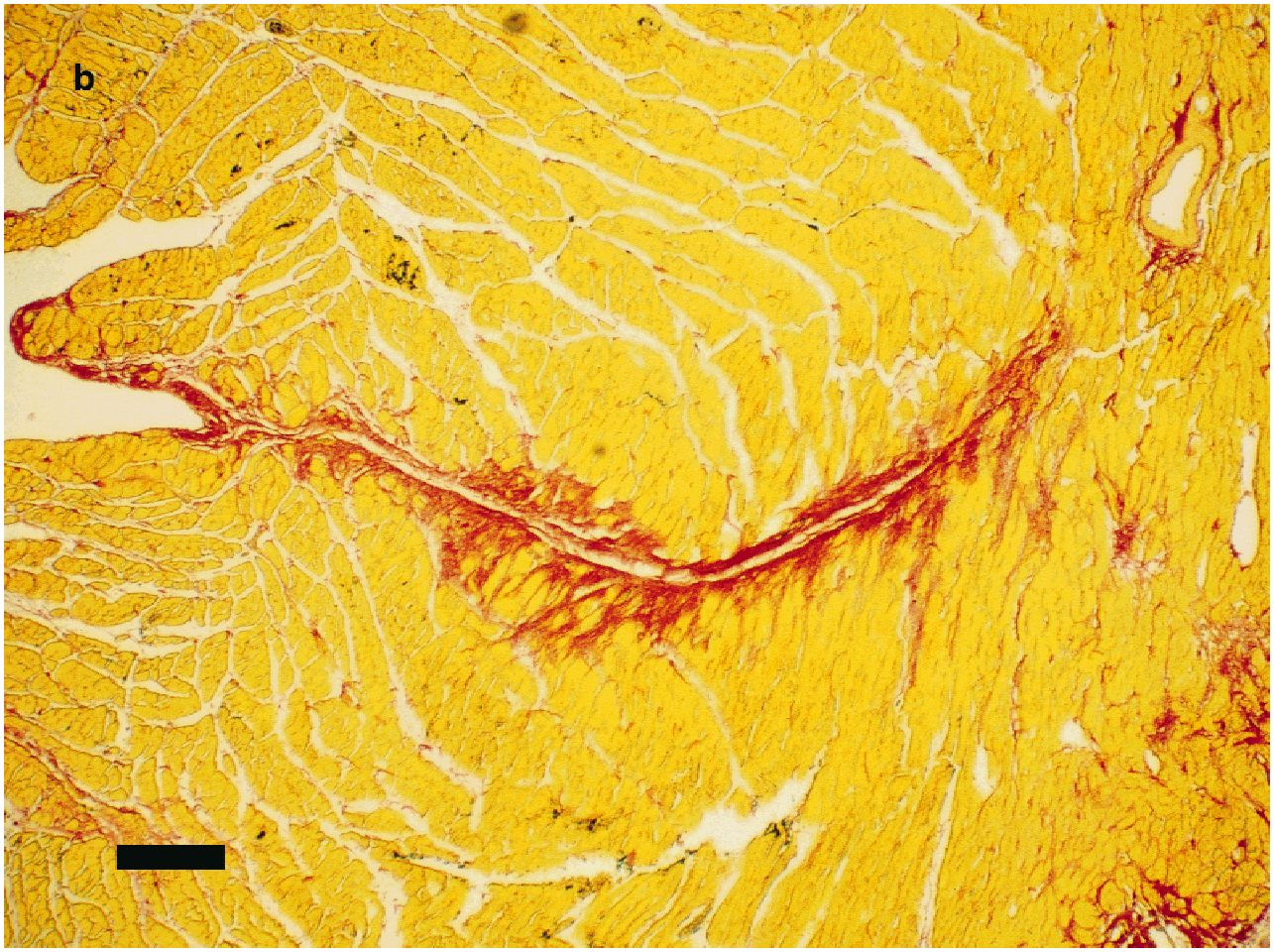

Fig. 1. (Continued) **b:** Limited amount of scar tissue. The left ventricular cavity is on the left in both panels. Scale bar = 200 μm.

9-nsec pulse width. The beam was focused into 400- and 600-μm diameter fibers.

Surgery

Ten female Sprague-Dawley rats (290–435 g) were anesthetized by an intraperitoneal injection of ketamine (50 mg kg^{-1}) and xylazine (10 mg kg^{-1}), intubated, and ventilated with room air. A thoracotomy was performed, and six transmural channels were made from the epicardial surface through to the ventricular cavity. All of the channels were located within the territory perfused by the left coronary artery. Transmural penetration was confirmed by bleeding from the channel, which was sometimes pulsatile. If bleeding did not soon stop, pressure was applied with a cotton-tipped swab until it did. The chest was then closed, and the rats were allowed to recover.

Between 51 and 165 days later, the rats were reanesthetized (as described above), a tracheostomy was performed, and the chest was reopened. The ability of the channels to supply blood to the heart was challenged by occlusion of the left coronary artery. A stitch was taken from the atrioventricular groove to the pulmonary cone to encircle the artery, which was occluded by tying a knot in the suture. After 90 min, the occlusion was released and reperfusion was continued for 4.5 hr. The artery was then reoccluded, and pigment (0.5 mL, Unisperse Blue) was injected into the circulation, so that the area not perfused by the artery appeared blue. The area normally perfused by the artery (called the area at risk) thus appeared pale. The hearts were then arrested by injection of potassium chloride, cut into 4–5 cross-sectional slices, photographed, incubated in triphenyltetrazolium chloride (TTC) for 10 min at 37°C, and rephotographed. TTC stains viable muscle red, whereas necrotic muscle does not stain and appears pale. We used planimetry to determine the

size of the area at risk (expressed as a percentage of the left ventricle) and the size of the area of necrosis (expressed as a percentage of the area at risk).

Histologic Analysis

Hearts were serially sectioned (7 μm thickness) and stained with picrosirius red, which stains muscle yellow and collagen red [6]. We examined three aspects of channel morphology: whether or not the channels were open, the width of fibrosis associated with each channel, and the structure of the fibrosis associated with each channel or channel remnant. To classify a channel as patent, there had to be an open connection to the left ventricular (LV) cavity and/or blue pigment within the channel. We adopted these criteria because Fisher and colleagues demonstrated that portions of the original channel can remain open in the absence of an actual conduit [7,8]. The presence of pigment within the channel was considered equivalent to an LV cavity connection because the lack of a native collateral circulation in rat hearts dictates that pigment would not be found within channels unless it had entered directly from the LV cavity. We used a calibrated eye-piece reticle to measure channel diameter and the scar width associated with each channel. The structure of the fibrosis was qualitatively assessed by examination of sections with polarized light, which enabled collagen fiber orientation to be visualized [9].

Statistics

Values are expressed as mean ± standard error. Means were compared with two-tailed t-tests. Values were considered different if the probability was less than 0.05.

RESULTS

Area at Risk and Infarct Size

There was no difference in the size of the area at risk between groups (low energy, 53 ± 2%; high energy, 55 ± 4%). Prolonged occlusion of the artery supplying blood to the region of the heart where the channels were made normally results in the appearance of a large transmural infarct. For the 90 min of coronary occlusion used in these experiments, approximately 65% of the area normally perfused by the occluded artery becomes necrotic [10]. However, if channels do supply blood to tissue made ischemic by arterial occlusion, the amount of necrosis will be reduced. Necrosis was less in hearts treated with high-pulse energy (24 ± 5% of the area at risk) than those treated with the low-pulse energy (56 ± 4%, $P < 0.05$).

Histology

The two pulse energies produced different amounts of injury and resulted in three types of channel: closed, open surrounded by wide bands of scar, and open surrounded by small bands of scar (Fig. 1). Connections to the ventricular cavity were identified for all open channels (Fig. 2), and vessels were often seen connecting to the channels. Channels created with the lower pulse energy had a patency rate of 46% and an average scar width of 220 ± 25 μm. Channels made with the higher energy had greater patency (89%, $P < 0.05$) and less scar (145 ± 10 μm, $P < 0.05$). The largest channel was 140 μm wide; however, the average diameter was smaller and did not differ between groups (low energy, 36 ± 11 μm; high energy, 33 ± 5 μm).

Although not all closed channels were associated with wide bands of scar tissue, we found differences in scar structure between open and closed channels. Collagen fibers immediately adjacent to every open channel were aligned approximately parallel to the long axis of the channel. In contrast, collagen fiber alignment was approximately perpendicular to this direction when the channels were closed (Fig. 3).

DISCUSSION

We found that laser-made channels can remain open for several months after they are made and that the structure of scar tissue associated with open channels differs considerably from that of closed channels. Furthermore, the ability of laser-made channels to provide protection against an ischemic challenge required that the channels not only be open but also that they be surrounded by a small amount of fibrosis.

Mechanism of Protection

The findings of open channels connected to the ventricular cavity and less necrosis in hearts treated with high-pulse energy were consistent with blood flow through the channels; however, additional supporting evidence was provided by histologic analysis. Blue pigment, injected into the circulation at the end of the experiments (with the artery reoccluded) to delineate the tissue nor-

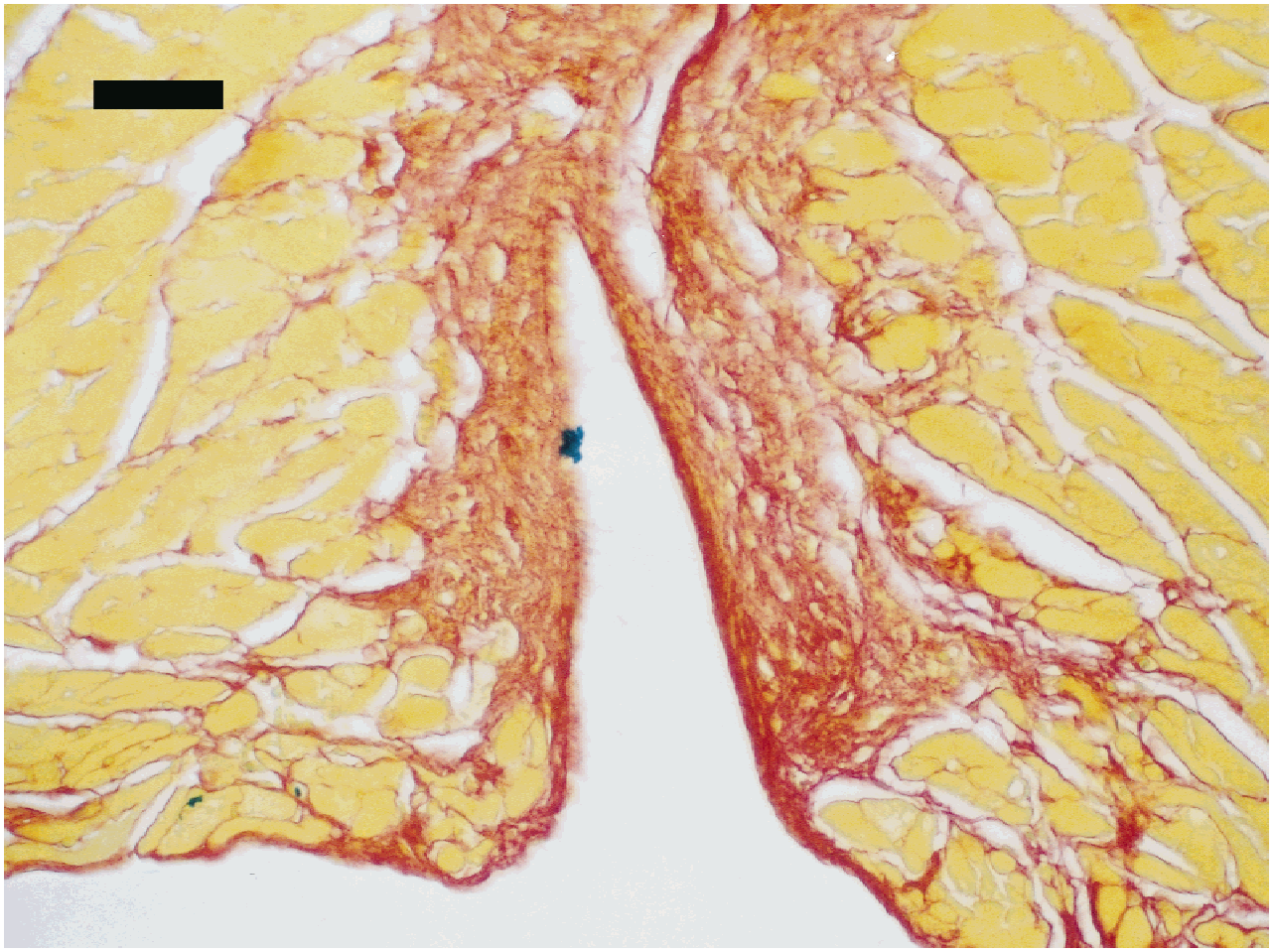

Fig. 2. Connection of a channel to the ventricular cavity. Blue pigment can be seen within the channel. Scale bar = 50 μm.

mally perfused by the artery, was found inside channels and in surrounding tissue located within the perfusion territory of the occluded artery (Fig. 2). The pigment could only have gained entry to these locations through direct connections to the ventricular cavity. In contrast, pigment was not seen in corresponding locations when channels were closed.

Vessels branching off open laser channels were observed. These connections provide potential links to the surrounding tissue. However, if extensive fibrosis surrounds the channel, these connections may terminate within the scar and thus never reach the surrounding muscle. This situation is illustrated in Figure 1a, where two channels made with the low-pulse energy are in close proximity. Pigment was seen within the channels and in the vascular connections but not in adjacent muscle. In addition, even though the channels were open, there was no reduction in necrosis. Thus, we propose that excessive fibrosis presents a barrier to flow.

Fibrosis as a Marker of Initial Injury

It is reasonable to suppose that the width of fibrosis measured several months after the channels were made provides an indication of the muscle necrosis caused by the channel-making process. Nevertheless, the correlation between necrosis and fibrosis may not be linear because scars are not passive structures but possess the ability to contract. This contraction is mediated by the tractional forces of both fibroblasts and actin filament-containing myofibroblasts and can reduce scar volume. Fisher et al. found that, although the necrosis associated with channels made with a holmium:YAG laser was greater than that associated with a carbon dioxide laser, scar size measured six weeks later was similar [7]. These results suggest that the greater the ini-

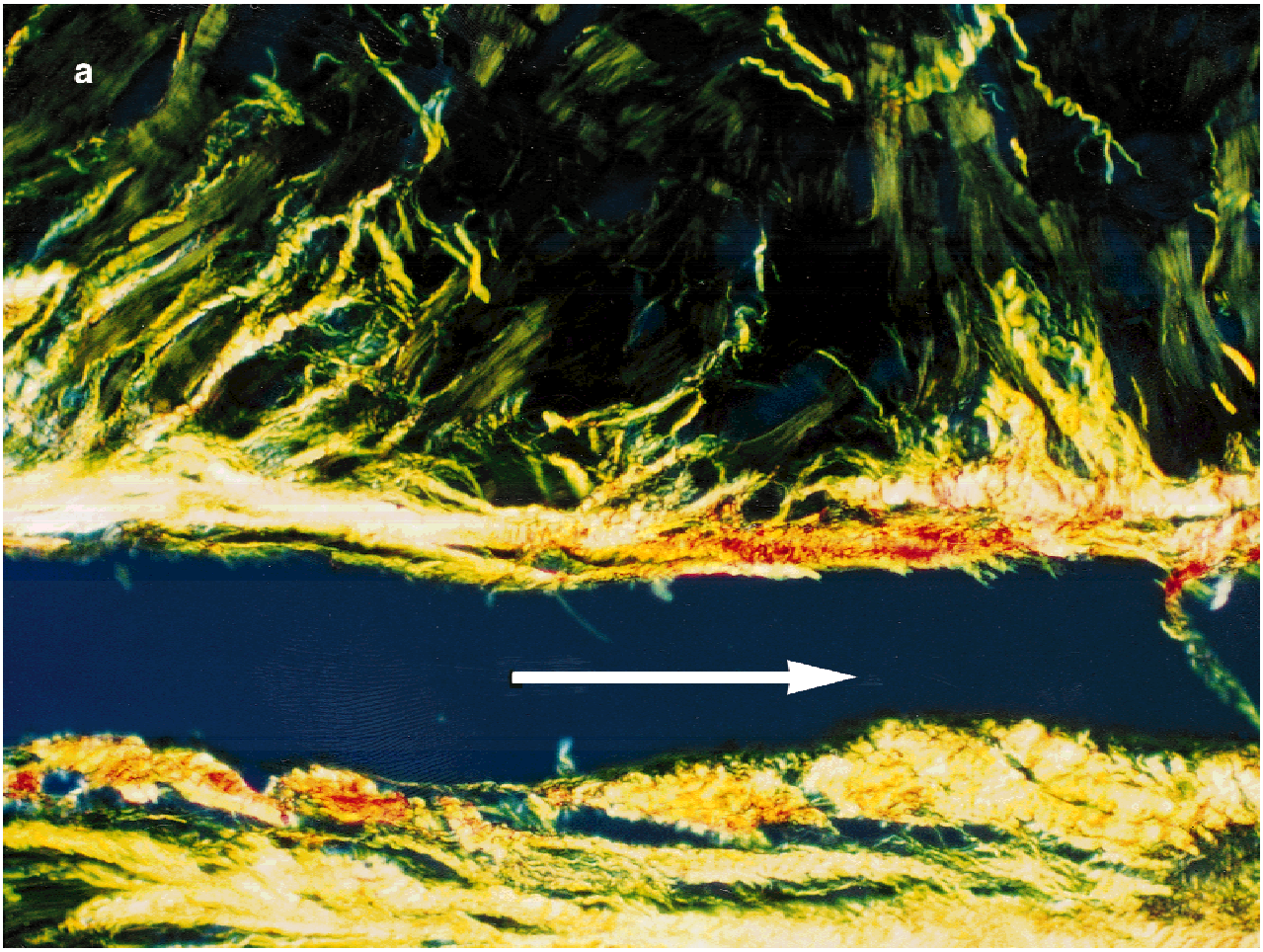

Fig. 3. Scar structure. Sections were stained with picrosirius red and viewed with polarized light. Collagen fibers appear yellow/orange and muscle apears green. **a:** Open channel. Collagen fibers are aligned parallel to the long axis of the channel (arrow) and perpendicular to adjacent muscle.

tial injury, the greater the subsequent contraction. Such contraction has been suggested to contribute to channel closure [11]. We propose that the channel patency in the present study reflects a smaller amount of initial injury than the injury caused by the infrared lasers mentioned above. However, collagen fiber organization in the scar tissue also appears to be important, but whether channels are open because of this structure or whether the structure develops because the channels are open is unknown. Further examination of scar structure is necessary to answer this question.

Mechanism of Channel Creation

Although the channels were able to provide protection under certain conditions, there is evidence that they were not created solely by ablation. It is customary to express laser "doses" in terms of energy density (fluence in J cm^{-2}) rather than in millijoules per pulse. However, when the data were divided on the basis of fluence, there was no correlation with infarct size, patency, channel diameter, or fibrosis. The failure to explain the data in terms of fluence suggests that the end result was not determined by this parameter. A similar failure of fiber diameter to explain the data rules out a purely mechanical mechanism, i.e., the channels were not created simply by pushing the fiber through the tissue. Instead, we propose that the channels were likely created by a combination of ablation and mechanical effects; in essence, a hot needle with an ability to remove some tissue. Whether the proposed combination of ablation and mechanical channel creation is superior or inferior to that of ablation alone is unknown. Nevertheless, it is clear that, whether because of scar contraction or inadequate

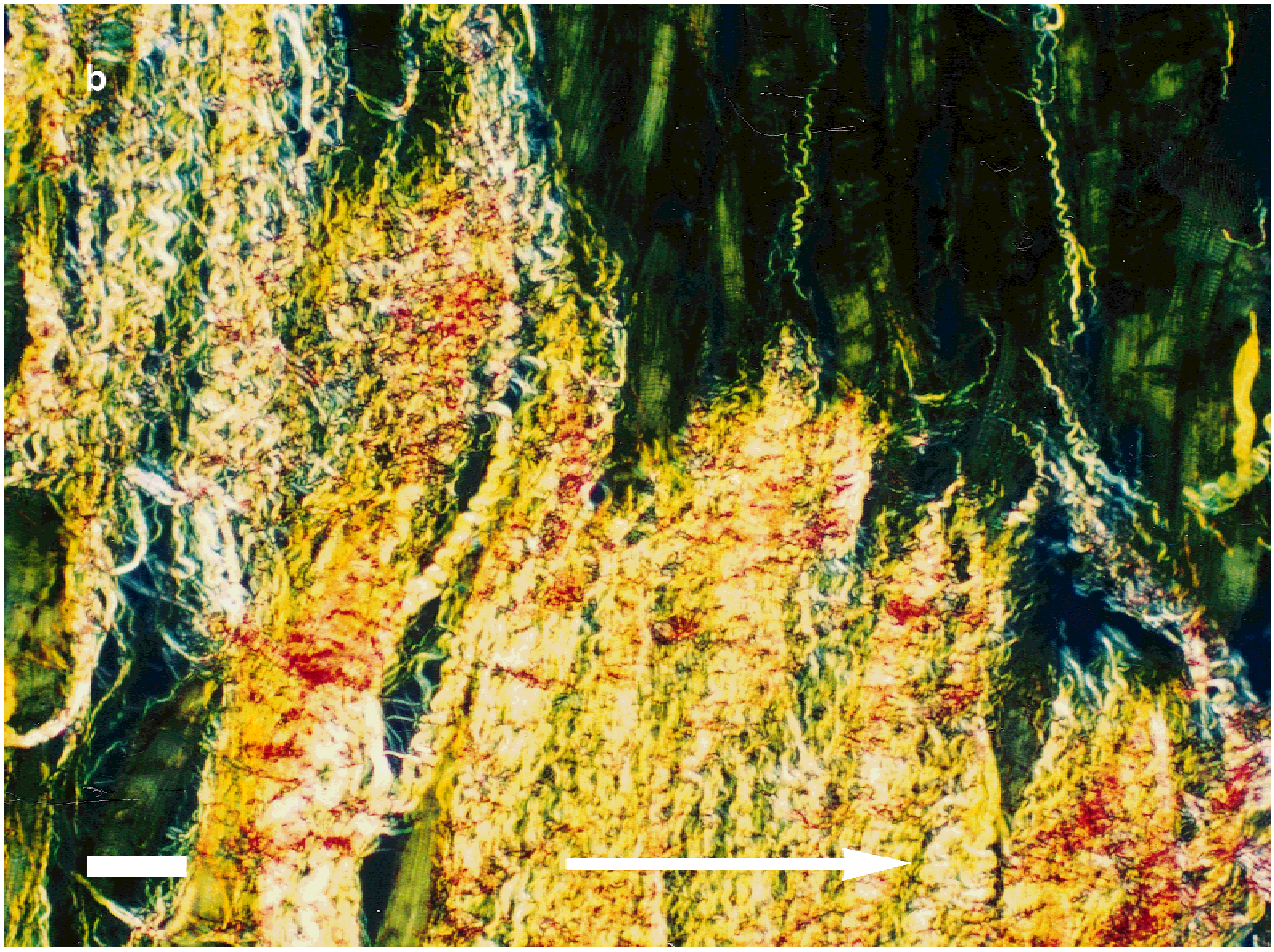

Fig. 3. (Continued) **b:** Closed channel. Collagen fibers are aligned perpendicular to the direction of the original channel (arrow) and parallel to adjacent muscle. Scale bar = 50 μm.

tissue removal, even the widest channel in our study was considerably smaller than the original fiber diameter.

Limitations

Our study evaluated only one laser system; thus, we do not know whether the same structural features are found after treatment with other wavelengths. However, the majority of studies using infrared laser treatment have reported that channels are eventually occluded by fibrosis [2,3,7,8,10]. Extrapolation of our results to clinical studies is complicated by our study design in which channels were made in healthy myocardium and an acute ischemic challenge was applied later. This approach was selected because of the lack of an animal model that adequately duplicates the clinical situation in which transmyocardial laser revascularization is used, i.e., hibernating myocardium. However, our results demonstrate that it is possible to create channels that remain open and thus provide a basis for human treatment.

Summary

Myocardial channels made with infrared lasers have been found to be closed by scar tissue within a few weeks [2,3]. Although reductions in angina have been found, evidence for improved blood flow to the treated tissue has been equivocal, probably because of channel closure. Our initial experiments in rat hearts with an ultraviolet laser indicate that a specific scar structure is required for channels to remain open and for open channels to protect against an ischemic challenge. The channels must connect to the LV cavity and adjacent myocardium and be surrounded by a small amount of scar in which collagen fibers are aligned parallel to the channel. Under such circumstances, laser treatment resulted in the cre-

ation of new conduits for blood flow. Thus, the method of channel making appears to be crucial to patency and efficacy.

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